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Award Number: DAMD17-99-1-9456

TITLE: Structure of the Tetrameric p53 Tumor Supressor Bound to DNA

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REPORT DATE: May 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 2000	Annual (15 Apr 99 - 14 Apr 00)

4. TITLE AND SUBTITLE
Structure of the Tetrameric p53 Tumor Supressor Bound to DNA

5. FUNDING NUMBERS
DAMD17-99-1-9456

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

The p53 transcriptional activator binds to DNA as a tetramer to activate the transcription of genes involved in cell cycle arrest and apoptosis, and alterations in the DNA-binding domain of p53 are the most common genetic changes found to date in breast cancer. The overall goal of the proposal is to determine the X-ray crystal structure of tetrameric forms of p53 bound to DNA. Over the last year we have made significant progress towards achieving this goal. Specifically, we have successfully cloned, overexpressed and purified to homogeneity two relevant protein constructs of p53 that are competent for tetramer formation on DNA; p53(98-292), and p53(86-351). We are pursuing the structure determination of both of these protein constructs bound to DNA in parallel. For the p53(98-292) protein construct we have obtained crystals of a p53/DNA complex and a structure determination is in progress, and for the p53(86-351) construct cocrystallization trials with DNA are in progress. The structure of these p53/DNA complexes will provide a mechanistic understanding into the structural basis underlying p53 mutations, and will provide a framework for the structure-based design of drugs that will be useful in the treatment of p53-mediated breast cancer.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
9

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

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5/9/00

PI - Signature Date

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(5) INTRODUCTION

Alterations of the p53 tumor suppressor gene are the most common genetic changes found to date in breast cancer, suggesting that the gene plays a central role in the development of the disease (Hollstein et al., 1994; Lee and Bernstein, 1995; Ozbun and Butel, 1995). p53 activity is mediated by a tetrameric form of the protein that binds to DNA to activate the transcription of genes involved in cell cycle arrest or apoptosis (Donehower and Bradley, 1993; Lee and Bernstein, 1995). The p53 protein contains four functionally distinct domains: an N-terminal transactivation domain, a central core DNA binding domain, a tetramerization domain, and a C-terminal regulatory domain (Pavletich et al., 1993; Wang et al., 1993). The vast majority of tumor-derived p53 mutations are localized to the core domain and thus prevent p53 from binding DNA (Hollstein et al., 1991). The overall goal of the proposal is to determine the X-ray crystal structure of a tetrameric form of p53 bound to DNA. The specific technical objectives of our proposal are to (1) Prepare p53 protein and DNA target sites for p53/DNA cocrystallization (tasks 1-4), (2) Crystallize the p53/DNA complex for structure determination using X-ray crystallography (tasks 5 and 6), (3) Determine the three-dimensional structure of a p53/DNA complex (tasks 7-13), and (4) Overexpress and purify to homogeneity the p53-T284R mutant protein that is able to rescue common tumor-derived p53-mutations, crystallize it bound to DNA, and determine the structure of the p53-T284R/DNA complex (tasks 14-20). The structure of the p53/DNA complex will provide a mechanistic understanding into the structural basis for how p53 mutations result in mammary carcinomas, and a comparison with the p53-T284R/DNA structure will provide a framework for the structure-based design of drugs that may mimic the T284R mutation (Wieczorek et al., 1996) and thus will be useful in the treatment of p53-mediated breast cancer.

(6) BODY

Over the last year we have made significant progress towards achieving the overall goal of the proposal. In our initial attempts to identify p53 molecules that were amenable to preparation for structural analysis, we screened p53 molecules from different species and have discover that p53 from mouse was the most amenable to biochemical and subsequent structural analysis. Specifically, we have successfully cloned, overexpressed and purified to homogeneity two relevant protein constructs of p53 that are competent for tetramer formation on DNA; p53(98-292) harboring the p53 core domain, and p53(86-351) harboring the core-linker-tetramirization region of p53 (task 1) (Figs. 1 and 2). We are pursuing the structure determination of both of these protein constructs bound to DNA in parallel. For the p53(98-292) protein construct we have progressed to task 7 of technical objective 3, and for the p53(86-351) construct we have progressed to task 5 of technical objective 2.

A more detailed analysis of our accomplishments follow. We have used gel-shift analysis to shown that both p53(98-292) and p53 (86-351) protein constructs are competent to bind an idealized p53 DNA target site as a tetramer (task3), and have also purified each of the 10 p53 DNA target sites outlined in our proposal for cocrystallization trials (task 2). A subset of the complexes showed monodisperse behavior when analyzed using dynamic light scattering and by gel filtration (task4) and these were set up for crystallization trails (task 5). We have not yet obtained protein/DNA complex crystals with the p53 (86-351) protein construct. For the p53(98-292) protein constructs, we have obtained crystals under several different crystallization conditions, and the crystals that diffract to the highest resolution when analyzed using a home X-ray source grow from a solution containing 0.2 mM p53/20-bp DNA, 8% PEG4000, 20 mM Mes, pH 5.6, 200 mM KCl and 50 mM$MgCl_2$ (task 6). We have collected a preliminary 3.0Å resolution data set from these crystals (Table 1) and analysis of the data indicate the crystals form in the space group $C222_1$ with unit cell dimensions of a=75.4 Å, b=121.3 Å, and c=185.5 Å (task 7) (Fig. 3). Consideration of the unit cell volume of these crystals suggests that they contain the p53 protein bound as a tetramer to one DNA duplex with about 50% solvent content in the asymmetric unit cell.

We are currently initiating the structure determination of the tetrameric p53(98-292)/DNA complex with the Molecular Replacement technique using the monomeric p53/DNA complex (Cho et al., 1994) as a search model. In parallel, we are preparing heavy atom derivatives of p53(98-292), including a seleno-methionine derivatized p53 protein, to obtain model-unbiased phases for the p53(98-292)/DNA structure. We are also arranging a synchrotron trip so that we can obtain higher resolution data (better than 3.0 Å) for the tetrameric p53(98-292)/DNA complex. As mentioned earlier, crystallization trials of the longer p53(86-351) construct bound as a tetramer to DNA are also underway.

(7) KEY RESEARCH ACCOMPLISHMENTS

- ❒ Overexpression and purification to homogeneity of a p53 core domain construct, p53(98-292).
- ❒ Overexpression and purification to homogeneity of a p53 (86-351) protein construct harboring the core domain, linker region and teramerization domain.
- ❒ Crystallization of a tetramer of p53(98-292) bound to a 20-bp DNA duplex.
- ❒ Data collection and characterization of crystals of the p53(98-292)/20-bp DNA complex.

(8) REPORTABLE OUTCOMES

No reportable outcomes to date.

(9) CONCLUSIONS

During the first year of the funding period we have made considerable progress towards achieving our proposed goals. Specifically, we have overexpressed and purified to homogeneity the p53(86-351) protein and cocrystallization efforts with DNA are underway (<u>through task 5 of technical objective 2</u>). In addition, we have obtained crystals and collected date from crystals of a p53(98-292) tetramer bound to DNA (<u>through task 7 of technical objective 7</u>).

By the end of the second year of the grant period we expect to be well on our way to completion of the structure determination of both the p53(98-292) and p53(86-351) protein constructs bound as tetramers to DNA (<u>technical objective 3, tasks 9-13</u>). These structures will set the stage for the completion of the final technical objective of the proposal (<u>technical objective 4</u>) during the final year of the proposal. The final objective of the proposal is to determine the structure of the DNA complex with the T284R p53 mutant protein that rescues tumor derived mutations. <u>A comparison of this structure with the native structures is expected to provide a framework for the structure-based design of drugs that may mimic the T284R mutation and thus will be useful in the treatment of p53-mediated breast cancer.</u>

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(11) APPENDICES

Figures 1, 2 and 3; and Table 1.

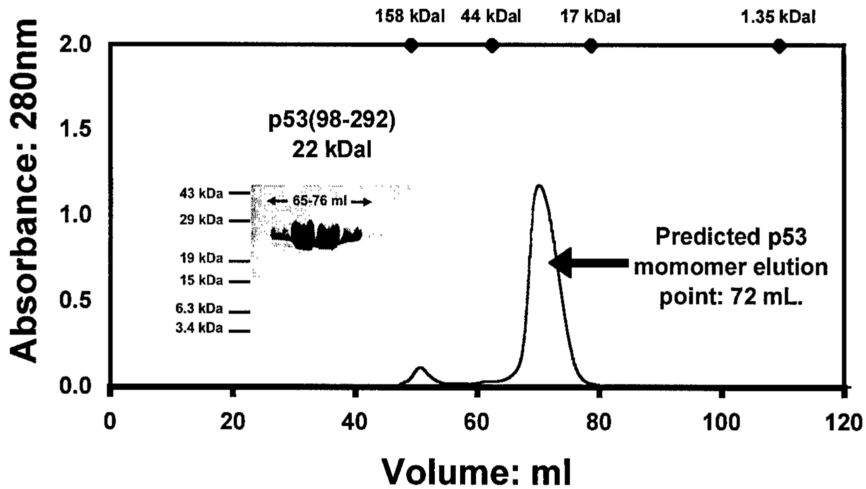

Figure 1. Purification of the p53(98-292) core domain. p53(98-292) was purified using a combination of cation exchange and gel filtration chromatography. The final gel filtration chromatograph on a Superdex-75 gel-filtration FPLC column is shown with the corresponding peak fractions illustrated on the embedded SDS-PAGE gel.

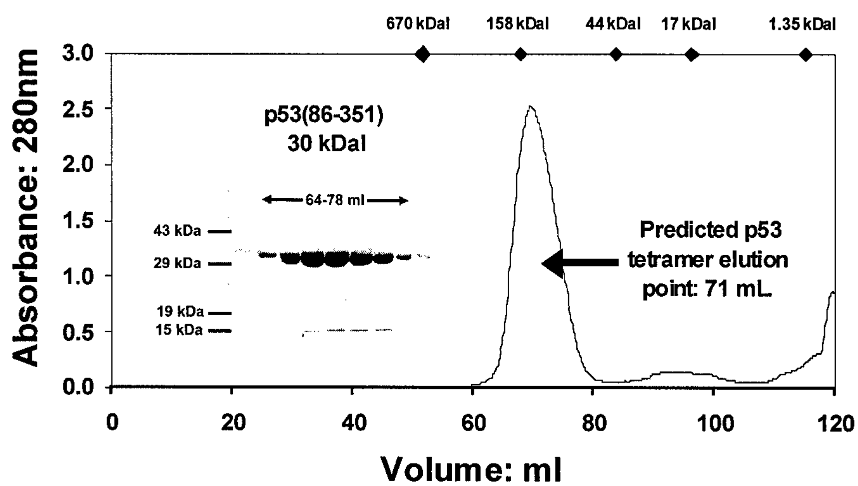

Figure 2. Purification of the p53(86-351) core-linker-tetramerization domain construct. p53(86-351) was purified using a combination of cation exchange and gel filtration chromatography. The final gel filtration chromatograph on a Superdex-200 gel-filtration FPLC column is shown with the corresponding peak fractions illustrated on the embedded SDS-PAGE gel.

A B

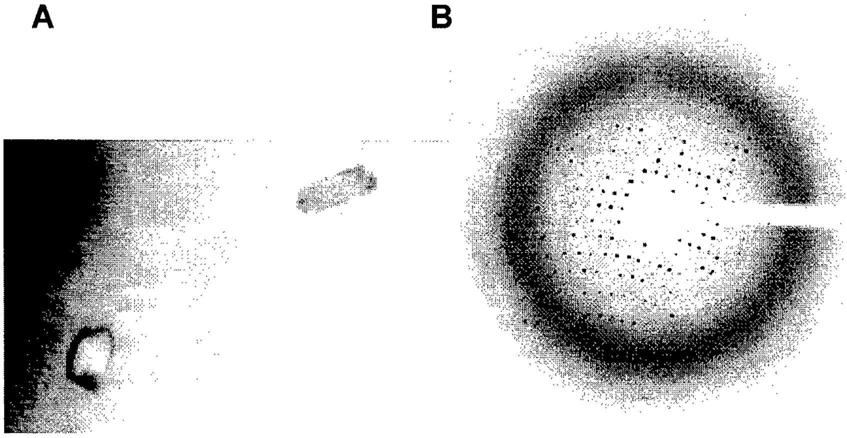

Figure 3. Crystals and diffraction from a tetrameric p53(98-292)/20-bp DNA complex. (A) Crystals of the complex are shown with the largest dimension of 0.5 mm. (B) Diffraction pattern from the crystals shown in (A). The highest resolution reflection occurs at 3.0 Å.

Table 1. Summary of crystallographic parameters for crystals of the tetrameric p53(98-292/20-bp DNA complex.

Parameter	$C2\ 22_1$
Cell parameters	a=75.38Å b=121.30Å c=185.45Å
no. of observations	70239
no. of unique reflections	31964
Tetramer per Asy. Unit	1
Vm (Å^3)	2.12
resolution (Å)	3.0
overall completeness (last shell)	97.2 (95.3) %
Overall I/sigma (last shell)	15.4 (2.3) %
*R*merge (last shell)	7.10 (32.3) %